



## THYROXINE (T4) CHEMILUMINESCENCE IMMUNOASSAY KIT

Catalog No. CL1002-2

### **INTENDED USE**

The Autobio T4 CLIA assay is designed for the quantitative determination of Thyroxine (T4) concentration in human serum.

### **INTRODUCTION**

Thyroxine or 3, 5, 3', 5'-tetraiodo-L-thyronine (T4) is the major hormone produced by the thyroid gland. It has a molecular weight of 777 daltons and is synthesized by iodination of tyrosine residues on thyroglobulin. Proteolytic cleavage of follicular thyroglobulin releases T4 into the bloodstream. Greater than 99% of T4 is reversibly bound to 3 plasma proteins in blood - thyroxine binding globulin (TBG) binds 70%, thyroxine binding pre-albumin (TBPA) binds 20%, and albumin binds 10%. Approximately 0.03% of T4 is in the free, unbound state in blood at any time.<sup>1,2</sup>

Diseases effecting thyroid function may present a wide array of confusing symptoms. Measurement of total T4 by immunoassay is the most reliable and convenient screening test available to determine the presence of thyroid disorders in patients. Increased levels of T4 have been found in hyper-thyroidism due to Grave's disease and Plummer's disease and in acute and subacute thyroiditis. Low levels of T4 have been associated with congenital hypothyroidism, myxedema, chronic thyroiditis (Hashimoto's disease), and with some genetic abnormalities.<sup>3,4,5</sup>

### **PRINCIPLE OF THE TEST**

In the T4 CLIA, a certain amount of anti-T4 antibody is coated on microtiter wells. A measured amount of patient serum, and a constant amount of T4 conjugated with horseradish peroxidase are added to the microtiter wells. 8-anilino-1-naphthalene sulfonate (ANS) is used to displace T4 from proteins to enable the measurement of total circulating T4. During incubation, T4 and conjugated T4 compete for the limited binding sites on the anti-T4 antibody. After 60 minutes incubation at 37°C, the wells are washed by Wash Solution. Upon the addition of the substrate, the horseradish peroxidase activity bound on the wells is then assayed by chemiluminescence reaction. The Related Light Unit (RLU) of the reaction is inversely related to the concentration of T4 in the test sample.

### **MATERIALS PROVIDED**

1. Antibody Coated Microtiter Plate: Microplate with anti-T4 Antibody coated wells (1 plate, 48 wells/96wells)
2. Enzyme Conjugate Reagent: Horseradish Peroxidase (HRP) labeled T4 in Stabilizing Buffer (1 vial, 6.0ml/11.0 ml)
3. Reference Standards: 0, 1.0, 2.5, 5.0, 15, 30µg/dl T4 in HEPES solution with preservatives. 1µg/dl of the reference standard is equivalent to 1µg/dl standard of the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP), P. R.C. (6 vials, lyophilized)
4. Substrate A: (1 vial, 3.5ml/6.0ml)
5. Substrate B: (1 vial, 3.5ml/6.0ml)
6. PBS-T Powder: PBS-Tween (1 bag, 5g)

### **MATERIALS NOT PROVIDED**

The following materials are required but not provided in the kit.

1. Distilled water
2. Precision pipettes for delivery of 20-200µl, 100-1000µl (the use of accurate pipettes with disposable plastic tips is recommended)
3. Luminometer
4. Vortex Mixer or equivalent
5. Washer for microplate
6. Quality control specimens
7. Incubator
8. Absorbent paper

### **STORAGE OF TEST KIT AND INSTRUMENTATION**

1. Unopened test kits should be stored at 2~8°C upon receipt. The test kit may be used throughout the expiration date of the kit (1 year from the date of manufacture). Refer to the package label for the expiration date.
2. Reconstituted standards should be used within 30 days and be frozen at -20°C for long term storage. Microtiter Plate after first use should be kept in a sealed bag with desiccants to minimize exposure to damp air. Other opened components will remain stable for at least 2 months, provided it is stored as prescribed above.

#### **SPECIMEN COLLECTION AND PREPARATION**

1. Serum is the recommended sample type for this assay. Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with test procedures and should be avoided.
2. Collect all blood samples observing universal precautions for venipuncture.
3. Allow samples to clot for 1 hour before centrifugation.
4. Avoid grossly hemolytic, lipemic or turbid samples.
5. Prior to use, specimens should be capped and stored up to 48 hours at 2~8°C. For longer storage, freeze the specimens at -20°C. Thawed samples must be mixed prior to testing.

#### **PRECAUTIONS AND WARNINGS**

1. For *in vitro* diagnostic use only.
2. Handling of reagents, serum specimens should be in accordance with local safety procedures.
3. All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.
4. Avoid any skin contact with all reagents.
5. Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

#### **REAGENT PREPARATION**

1. All reagents should be brought to room temperature (18-25°C) prior to use.
2. Adjust the incubator to 37°C
3. Reconstitute each lyophilized standard with 0.5 ml distilled water. Allow the reconstituted material to stand for at least 10 minutes. Reconstituted standards should be stored sealed at 2~8°C.
4. Prepare Wash Solution: Add 1 bag of PBS-T Powder to 500 ml of distilled water, and mix well. The wash solution is stable at room temperature for two months.

#### **IMPORTANT NOTES**

1. Do not use reagents after expiration date.
2. Do not mix or use components from kits with different lot numbers.
3. It is recommended that no more than 32 wells be used for each assay run, if manual pipette is used, since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipette is available.
4. Replace caps on reagents immediately. Do not switch caps.
5. The wash procedure is critical. Insufficient washing will result in poor precision and invalid results.

#### **ASSAY PROCEDURE**

1. Secure the desired number of coated well in the holder. Make data sheet with sample identification.
2. Dispense 50µl of standards, samples, and controls into appropriate wells.
3. Dispense 100µl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is important to have complete mixing in this step.
5. Incubate at 37°C for 60 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and flick the microtiter plate 5 times with wash solution.
8. Strike the plate sharply onto absorbent paper to remove residual water droplets.
9. Dispense 50µl of Substrate A, then 50µl of Substrate B into each well. Gently mix for 10 seconds.
10. Put the microtiter plate into the detecting chamber of Luminometer for 5 minutes, then read the RLU values of each well.

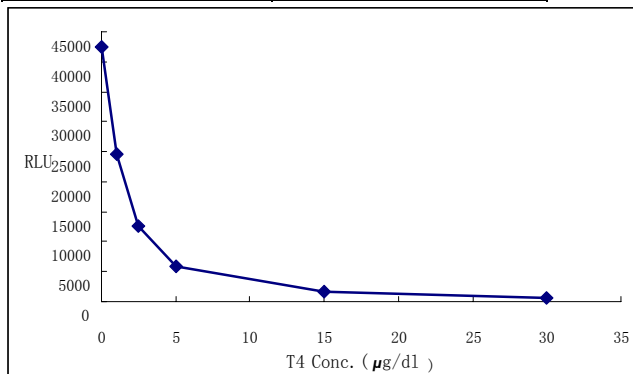
#### **CALCULATION OF RESULTS**

1. Calculate the mean value from any duplicate reagents. Where appropriate, the mean values should be used for plotting.
2. On linear graph paper plot the RLU (ordinate) for each reference standard against the corresponding concentration of T4 in  $\mu\text{g/dl}$  (abscissa) and draw a calibration curve through the reference standard points by connecting the plotted points with straight lines.
3. Read the concentration for each control and sample by interpolating on the calibration curve.
4. Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.

**EXAMPLE OF STANDARD CURVE**

A typical standard curve shown below is for the purpose of illustration only, and should never be used instead of the real time calibration curve.

T4 ( $\mu\text{g/dl}$ )	RLU
0	42572.9
1	24617.2
2.5	12632.5
5	5783.5
15	1643.1
30	694.8



**EXPECTED VALUES**

A normal range of  $5\mu\text{g/dl}$  to  $13\mu\text{g/dl}$  (central 95% interval) was obtained by testing serum specimens from 187 individuals determined as normal by Autobio TSH CLIA kit and Autobio Total Thyroxine (T4) CLIA kit. It is recommended that each laboratory establish its own normal range

**PERFORMANCE**

**A. Sensitivity**

Twenty zero standards were assayed along with a set of other standards. The sensitivity, defined as the apparent concentration corresponding to two standard deviations below the average RLU at zero binding, was lower than  $0.4\mu\text{g/dl}$ .

**B. Specificity**

The cross-reactivity of the T4 assay kit with T3 and rT3 was determined by adding these hormones to zero standards. The RLU produced was then determined.

Interferent	Concentration	Measured Value ( $\mu\text{g/dl}$ )	Crosstalk Rate (%)
T3	500ng/ml	1.08	2.16
rT3	500ng/ml	0.78	1.56

**C. Precision**

**a. Intra-Assay Precision**

Intra-Assay Precision was determined by assaying 20 replicates of each of 2 sera; low and high.

Serum	Number	Mean	SD	RSD (%)
Low	20	3.94	0.23	5.83
High	20	11.23	0.42	3.74

**b. Inter-Assay Precision**

Inter-assay Precision was determined by assaying duplicates of 2 serum pools in 20 separate runs, using a standard curve constructed for each run.

Serum	Number	Mean	SD	RSD (%)
Low	20	3.87	0.33	8.53
High	20	10.85	1.05	9.68

**D. Accuracy**

For 90 samples in the range of 1.5µg/dl to 25µg/dl, the relationship between the Autobio T4 CLIA Test and the Biocheck® T4 ELISA Test is described by the equation below:

Reference	No. of Specimens	Least Square Regression Analysis	Correlation Coefficient
Biocheck® (ELISA)	127	$Y=1.1321x + 0.9367$	0.957

**LIMITATIONS**

Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.

Serum T4 concentration is dependent upon a multiplicity of factors: hypothalamus gland function and its regulation, thyroxine binding globulin (TBG) concentration, and the binding of T4 to TBG. Thus, total T4 concentration alone is not sufficient to assess clinical status.

Heterophilic antibodies in human serum can react with reagent immunoglobulin, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference thus anomalous values may be observed. Additional information may be required for diagnosis. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

**QUALITY CONTROL**

Good laboratory practice requires that quality control specimens be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges. Controls containing sodium azide should not be used.

**SYMBOLS**

	BATCH CODE
	USE BY
	MANUFACTURER
	CONTAINS SUFFICIENT FOR <n> TESTS
	IN VITRO DIAGNOSTIC MEDICAL DEVICE
	TEMPERATURE LIMITATION
	CATALOGUE NUMBER
	CONSULT INSTRUCTIONS FOR USE

**REFERENCES**

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- 3) Surk M L, Chopra I J, Mariash C N. et a1. American thyroid association guidelines for use of laboratory tests in thyroid disorders [J]. JAMA, 1990, 263: 1529—1532.
- 4) Glinoe D. The regulation of thyroid function in pregnancy: path ways of endocrine adaptation from physiology to pathology [J]. Endocrinol Rev. 1997. 18: 404—33.
- 5) Demem L M, Spencer C A. Laboratory medicine practice guidelines for laboratory support for the diagnosis and monitoring of thyroid diseases [J]. National Academy of Clinical Biochemistry, 2002.

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